THE EFFECTS OF ULTRA-VIOLET RADIATIONS OF VARIOUS WAVE-LENGTHS UPON CLEAVAGE OF SEA URCHIN EGGS

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In a preceding paper retarding effects of radiations of λ2537A upon cleavage of eggs of the sea urchin, *Strongylocentrotus purpuratus*, were reported (Giese, 1938). The following account, extending the above study to wave-lengths of 2654, 2804, 3025, 3130 and 3660A, attempts to determine (1) the amount of retardation, if any, produced by known dosages of the above wave-lengths; (2) the amount of acceleration, if any, following low dosages; and (3) the region of the spectrum most effective in producing the effects noted.

The source of radiations (a water-cooled quartz mercury arc), the monochromator with optical parts of natural quartz, and the thermo-pile-galvanometer system for measuring intensities—were the same as those employed in the previous study. The data on intensities and the dosage ranges employed are summarized in Table I.

The animals were collected during the winter breeding seasons of 1937 and 1938 and the eggs were obtained and handled in the manner previously described. Approximately 300 eggs were used for each test. The experiments here reported were performed on fertilized eggs, irradiation beginning approximately 30 minutes after insemination. A dosage series of approximately 1024, 256, 64, 16 and 1 second was given at each of the wave-lengths. Three series of experiments were performed at each wave-length.

In most cases eggs from a single female were irradiated with a series of graded dosages of a given wave-length. In a few cases eggs from a single female were used for a series of dosages at each of two different wave-lengths. Thus in one instance eggs from one female were used for experiments with radiations of both λ2654 and λ2804A. The differences in effects of irradiation in this and other comparable cases were similar to those obtained with eggs of different females, the differences being of second order.

Following irradiation the eggs were pipetted into 1″ Syracuse watch-glasses, approximately 100 per dish, and were kept at 15 ± 1° C. Examinations were made at half-hour intervals, an attempt being

made to obtain the time when a large proportion of the eggs had just completed a given cleavage. After the 64-celled stage had been attained observations were made every 12 hours. As in the previous study, so here, abnormal development of eggs was found to be only a small part of 1 per cent of the total number used. Controls always developed into plutei normal to all appearances, therefore the conditions were considered satisfactory for the investigation.

TABLE I

Intensity and dosage measurements

λ in A	Exper. Number	I in ergs/sec./mm.²	Maximum dosage failing to retard cleavage in ergs/mm.²	Total dosage range in ergs/mm.²
2537	1	1.24	37.2	1.07–1585
	2	1.15	34.5	
	3	1.07	32.1	
2654	1	3.19	—	2.76–3444
	2	2.84	45.45	
	3	2.76	44.2	
2804	1	2.86	—	2.80–3090
	2	2.86	—	
	3	2.80	—	
3025	1	4.06	32.5	4.06–6840
	2	5.10	81.6	
	3	5.10	—	
3130	1	20.7	166	11.48–21,200
	2	11.48	—	
	3	13.74	210	
3660	1	38.5	(39,400)*	27.7 –74,500
	2	27.7	28,300	
	3	44.3	(74,500)*	

* At these dosages retardation was observed, but too slight to be accurately determined.

EXPERIMENTAL

In none of the experiments for any of the wave-lengths and dosages tried was there indication of definite stimulation and acceleration of the division rate, a finding in agreement with previous studies at λ2537A. The minimum dosages used are given in Table I. The eggs irradiated with a dosage below the retardation threshold cleave comparably to controls within the limits of accuracy of the investigation.

It is, of course, possible that the exact dose required for stimulation was not given or that even the lowest dosage was too great to induce acceleration.

While at the smaller dosages there was no retardation of the cleavage rate, such retardation was evident with sufficiently large dosages, the action of different wave-lengths being different only in degree. In each case retardation did not occur below a certain threshold (Fig. 2). In Table I are given data on the maximum dosage tried which failed to retard cleavage.

Retardation of cleavage was found to increase with dosage at all of the wave-lengths, as might be expected. After intermediate dosages of radiation, cleavage was normal and rhythmic, but after large dosages not only was cleavage delayed but it was often irregular; for example, whereas controls showed at most two cleavage stages at any given time, those irradiated with large dosages might show three or even four stages at one time. Occasionally abnormal cleavages appeared as well. Therefore it was often difficult to collect data for the larger dosages. None of the dosages used, however, were adequate to completely prevent cleavage.

Retardation following prolonged irradiation was apparent not only during cleavage but also at later stages in development. Thus at the time the blastulae of the controls were rotating within the fertilization membranes, those sufficiently irradiated were still immotile; when controls hatched from the membranes, those irradiated were still confined; when gastrulae formed, those irradiated were only beginning invagination.

In all cases eggs irradiated for 1, 16, and 64 seconds gave rise to blastulae, gastrulae and plutei normal to all appearances; in most cases those irradiated for 256 seconds did so as well. However, only in experiments at wave-lengths of 2537, 3130 and 3660A did plutei develop when eggs were given the maximum dosages recorded in Table I. In the experiments with large dosages at other wave-lengths the blastulae were as a rule abnormal and gastrulated abnormally. When plutei-like larvae developed they were abnormal, although in some instances skeletal rods, differentiated gut and pigment cells appeared. Many of these eggs, however, developed into balls of motile cells which failed to develop further. When in other cases blastulae and gastrulae were formed, however, they were choked with cells and appeared much darker than controls.

For each experiment the number of divisions of the controls and irradiated eggs was plotted against the time since insemination as in

Fig. 1, an example of a dosage series at λ2804A. The dosage in ergs per square millimeter for each curve was determined from the intensity readings and added to the graphs. The difference in time of development to the 8-celled stage between the control and the irradiated eggs was determined from the graph. The retardation of this third cleavage was then plotted against the log of the dosage (in ergs/mm.^{2}) for each of the wave-lengths to obtain a comparison of the efficiency of the various wave-lengths in retarding cleavage. The data are given in

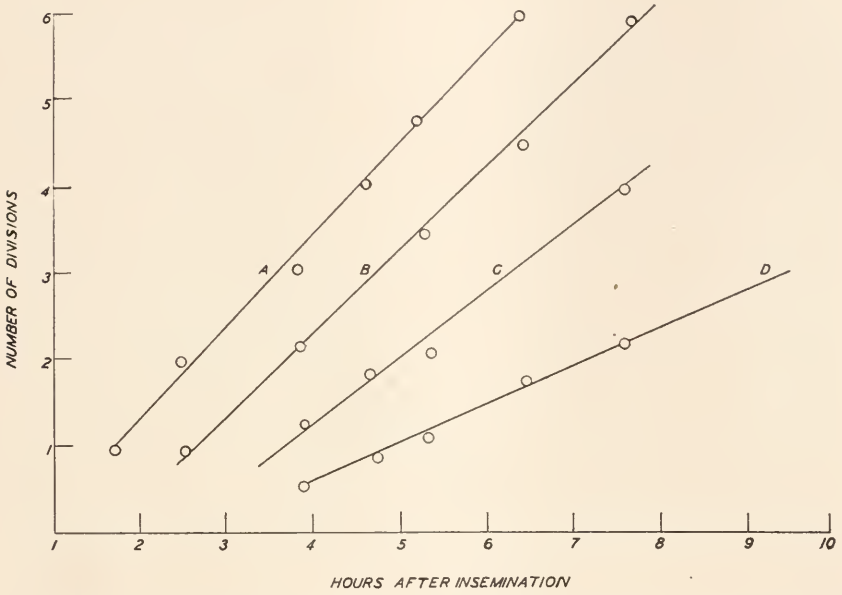

Fig. 1. Retardation of cleavage by irradiation with λ2804A, intensity 2.86 ergs/mm.^{2}/sec.

A. Control.

B. Dosage 183 ergs/mm.^{2}; retardation at 8-cell stage 1 hour and 8 minutes.

C. Dosage 1244 ergs/mm.^{2}; retardation at 8-cell stage 2 hours and 42 minutes.

D. Dosage 3090 ergs/mm.^{2}; retardation 5 hours and 48 minutes.

Fig. 2. The third cleavage was chosen for the comparison because (1) it is the midpoint of the developmental curves obtained; (2) it can be determined with greater accuracy than later cleavages; (3) it probably occurs after the latent period lag and by the time the effects of the irradiation have had full opportunity for expression. While the data are fairly homogeneous there is variability as detected in the spread of points on each side of the curves. It is possible that eggs from different females react to variable degrees. Part of the variation is undoubtedly

due to temperature changes and temperature differences for different experiments ($T = 15 \pm 1°$ C.). It is possible that minor errors in estimates and in other experimental procedures add further to the spread.

Examination of Fig. 2 discloses that the order of efficiency of the wave-lengths used, on the basis of incident energy, from the most effective to least is (1) 2804A, (2) 2537, 2654, 3025, (3) 3130 and (4) 3660. The difference between (1) and (2) is not large; the difference

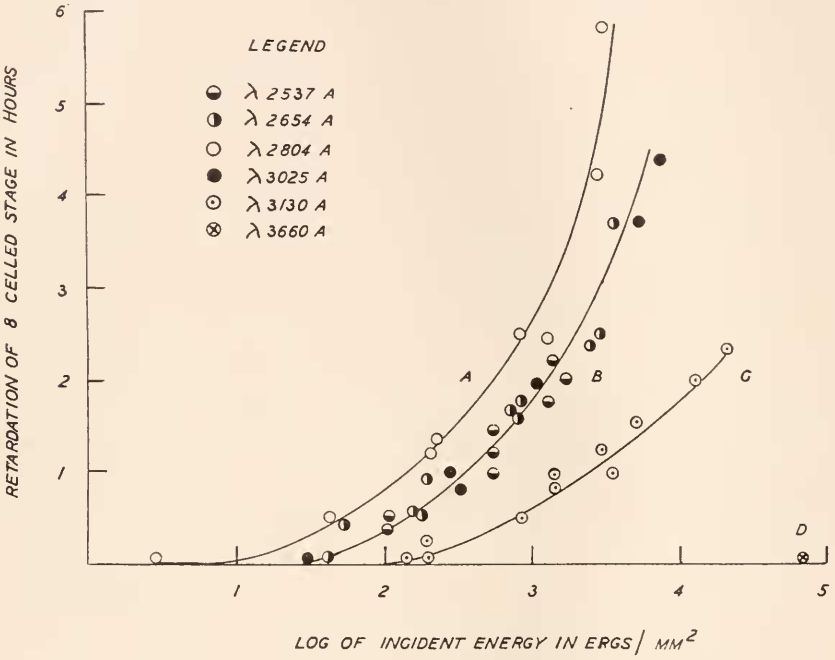

FIG. 2. Difference in efficiency of various wave-lengths in retardation of cleavage.

A. Curve drawn through points for λ2804A.
B. Curve drawn through points for λ's 2537, 2654, and 3025A.
C. Curve drawn through points for λ3130A.
D. λ3660A.

between (2) and (3), however, is great and between (3) and (4) very great. The difference in efficiency at the different wave-lengths may be due to the differences in absorption at the various wave-lengths. According to the Grotthus Draper law, only absorbed light is effective in promoting a photochemical reaction. If there is to be exact proportionality between absorption and effect, we should expect that the absorption at λ2804A would be about double that at λ2537, 2654 and 3025A and about 20 times that at 3130A.

Vlès and Gex (1928, 1934), using a quartz microscope and a spectroscope, measured the extinction of light by single eggs of the sea urchin, *Paracentrotus lividus*. They found for the fresh unfertilized egg a strong absorption band below λ2900A with a maximum at about λ2800A. At λ2537 and λ2654A the absorption was almost as great (the difference being of the order of one per cent), at λ3025A it had fallen off some 20 per cent, and only a little more to the beginning of the visible where another drop occurred. The fertilized egg was observed to have an absorption spectrum similar in general to that of the unfertilized egg (1928). Considerable variation was noted from egg to egg.

Unfortunately comparable equipment was not available to check the extinction by the eggs here used. However, the method previously used for paramecia was employed (Giese and Leighton, 1935*b*), readings of the galvanometer being taken first with the cell empty, then filled with unfertilized eggs, and the eggs counted. Three and in some cases four or five measurements were made at each wave-length, after several preliminary trials in which the eggs were not counted, and the averages are given in Table II. Considerable variation was observed for different samples of eggs. While the experimental error is large, as indicated by the standard deviations, the order of magnitude of the extinction is indicated, and such large differences as between λ2537 and λ2804A are significant. In all cases the differences of the averages of the groups from one another are many times the probable error, which is generally less than ± 0.5. In contrast to the data of Vlès and Gex, these data indicate a maximal extinction at λ2537A. Vlès and Gex found in most cases a decrease in absorption by normal eggs at this wave-length although in some of their cases this was not true. For eggs undergoing cytolysis due to dilution of sea water with distilled water they found a rise at λ2537A. That the method here used is not without merit is indicated by measurements on extinction of the ultra-violet light by sea urchin sperm. It was found that the maximum extinction occurred at λ2654A, a considerable decrease occurring at λ2804A, which is well in agreement with what might be expected of a cell made up mainly of nucleoproteins. However, it must be remembered that only part of the extinction is due to true absorption, the rest being due to scattering. With the experimental method here used the scattering would probably be a greater source of error than with the method employed by Vlès and Gex. Scattering varies inversely with some power of the wave-length as well as with the change in refractive index with wave-length (Teorell, 1930). If the change in the refractive index were negligible and the approximate value of the change in

scattering with wave-length found for *Paramecium* were to be used here (Giese and Leighton, 1935*b*), one could not explain the high extinction value at λ2537A as due entirely to a higher scattering. On the other hand, it is possible that the refractive index changes suddenly and that a corresponding increase in scattering occurs. The high value at λ2537A may, however, indicate a high absorption. Gates (1930) observed a higher absorption value at λ2537A than at λ2804A for both *S. aureus* and *B. coli*.

It is probable that in both sets of data the scattering masks the true absorption, but it is unlikely that the scattering is so great as to completely disguise differences of the order of magnitude needed to explain completely the differences in action of the different wave-lengths. It therefore seems more probable that it is not total absorption but rather selective absorption by some sensitive material which determines

TABLE II

Extinction of ultra-violet light by sea urchin eggs

λ in A	$1 - I/I_0 \times \frac{1}{A_e} \times 100$	λ in A	$1 - I/I_0 \times \frac{1}{A_e} \times 100$
2537	105 ± 5.50	3130	64.73 ± 8.00
2654	71.3 ± 4.40	3660	64.53 ± 9.32
2804	76.3 ± 7.78	5844	41.17 ± 4.21
3025	76.06 ± 5.54	5904	25.02 ± 2.44

I_0 = intensity of incident light, I of transmitted light.
A_e = fraction of the cell bottom occupied by eggs.
The whole expression gives the percentage of light incident upon the egg which is extinguished.

destructiveness of the rays. On this basis the substance absorbing λ2804A is most likely the sensitive substance, for approximately equivalent absorption is found at λ2654 and λ3025A, but their efficiency is less than that of λ2804A. The low efficiency of λ2537A may be due to the fact that radiations of this wave-length are so strongly absorbed at the exterior of the cell that they do not reach the seat of the sensitive material (Sonne, 1929).

Considering the high extinction values, the efficiency of λ3130 and λ3660A is remarkably low. At λ3130A the efficiency is about one-eighth that at λ2537, λ2654 and λ3025A. At λ3660A it is exceedingly small, as even after a dosage of 74,500 ergs/mm.^{2} the eggs showed such slight indications of retardation that accurate measurement of the delay in cleavage could not be made.

Discussion

The failure to obtain acceleration by irradiation with either the lethal region in dosages below the retarding dose or by the non-lethal region of the quartz ultra-violet indicates that either (1) this part of the spectrum is ineffective for acceleration, (2) the dosage range was not the proper one, (3) sea urchin eggs are unfavorable material. It is possible that the sea-urchin eggs are already cleaving at a maximal rate and so the normal cleavage rate cannot be significantly accelerated at a given temperature.

The low retarding efficiency of the long wave-lengths of ultra-violet, 3130 and 3660A, is not surprising on the basis of previous investigations (Gates, 1929; Oster, 1934; Giese and Leighton, 1935*a*) which have indicated a very low efficiency of λ3130A in destroying cells. Wyckoff (1932) has shown that 47 times as much energy is necessary to kill 50 per cent of the bacteria at λ3132A as at λ2654A. The λ3660A is generally considered beyond the region of destructive action. On the other hand, Coblentz and Fulton (1924) consider this region in very high dosages bactericidal, and Hertel (1905) maintained that even at λ4400, 5230 and 5580A retardation of division of sea urchin eggs was obtained when the intensity was sufficiently great.

The high efficiency of λ2804A, whatever the absorption may be, is, however, surprising in view of the many investigations in which λ2654A has been found more effective as a bactericidal (Gates, 1929; Wyckoff, 1932; Ehrismann and Noethling, 1932; and Hollaender and Claus, 1936) and a photolethal agent (Oster, 1934, and Ehrismann and Noethling, 1932, on yeasts; Weinstein, 1930, on paramecia, and Mayer and Schreiber, 1934, on tissue culture). On the other hand, λ2800A has been observed to be more effective than λ2654A in a number of instances (Lassen, 1928; Sonne, 1929; and Giese and Leighton, 1935*b*, on paramecia and Ehrismann and Noethling, 1932, on *B. prodigiosus*).

Since λ2800 is near the middle of the strong absorption band (2700-2850A) of serum and egg albumin (Coulter, Stone and Kabat, 1936) while λ2654 is near the midpoint of strong absorption by thymus nucleic acid and some nucleoprotein derivatives (Heyroth and Loofbourow, 1933) a decision between the two is of importance to the theory of the mode of action of the radiation. Gates (1929) and others following him have postulated that since maximum efficiency in lethal action on bacteria is in the vicinity of λ2654A and since this is the seat of the maximal absorption by nucleoproteins the lethal effect is mediated by absorption of the radiations by the sensitive nucleoproteins. For retardation of the eggs, however, it seems as if the region of maximal effi-

ciency is that absorbed by the proteins of the cell other than nucleoproteins. This suggests that retardation of cleavage may be brought about not so much by an effect upon the nucleus as by interference with some part of the cleavage mechanism. The relative inefficiency of λ2537A which is absorbed strongly by the surface would tend to exclude the surface as the only or the major seat of action. Just which part is affected remains for further experimentation to discover.

Summary

1. The effects of a series of dosages of known intensities of monochromatic quartz ultra-violet light upon fertilized eggs of the sea urchin *Strongylocentrotus purpuratus* were studied.

2. Even at the highest dosages of λ3660A used the eggs cleaved at just about the natural rate of controls; λ3130A produced more pronounced retardation of cleavage; λ2537, λ2654 and λ3025A were about equally effective, and λ2804A most effective of all.

3. No signs of definite and marked acceleration were observed at any of the wave-lengths for any of the dosages employed.

4. The destructive effect may be mediated by some substance most sensitive to λ2804A.

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